

EFFECT OF SILVER NANO-PARTICLES AND MYCORRHIZAE SYMBIOSIS ON THE DEVELOPMENT OF ARTICHOKE

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ABSTRACT

A field experiment was conducted for two successive seasons on artichoke (*Cynara scolymus* L.) treated with either the commonly used anti-fungal stumps, AF or nano-silver, NS using two sources of P i.e. rock phosphate, RP and the traditional chemical calcium super phosphate, SP under inoculation with or without Vesicular Arbuscular Mycorrhizas (VAM) (*Glomus mosseae*). Results revealed that VAM improved the effectiveness of the RP treatment and that the treatment RP + VAM recorded the highest plant growth, and total yield beside of improving the quality of heads than those attained by SP + AF. On the other hand, pre-planting with NS resulted in vigor growth, higher yield and head quality compared with AF. Thus, using RP + VAM + NS is the recommended practice to attain good growth parameters and achieve early and high total yield with better quality of heads. The increases in yield resulted from using this particular treatment were about 20.3 and 18.6 % as an average in both seasons for total yield per plant and per feddan, respectively, higher than the corresponding ones resulted from using SP + AF in the two growing seasons.

KEYWORDS: Globe Artichoke, Vesicular Arbuscular Mycorrhizae (VAM), Nano-Silver (NS), Anti-Fungai (AF), Rock Phosphate (RP), Calcium Super Phosphate (SP)

INTRODUCTION

Phosphorus (P) is one of the limiting factors for crop production; however, undergo rapid fixation in the Egyptian soils upon application in the form of soluble fertilizers i.e. superphosphate (El-Katkat, 1992). Mycorrhiza is one of the promising bio-techniques that can be used effectively for increasing P-availability in soil and improving its uptake by plants, particularly the perennial crops like artichokes (Ezz El-Din *et al*, 2010). Vesicular Arbuscular Mycorrhizae (VAM) are the main group of mycorrhiza fungi which improve plant growth through different mechanisms such as phosphate solubilization and enhancement of water / nutrients absorption (Rai, 2006). This ability of VAM is mainly attributed to its hyphae. Hyphae are external root shaped organs which penetrate soils and cracks, and make higher soil volume available to plant to be used as the source of water and nutrients (Wiedenhoeft, 2006). Different experiments have reported the improvement of plant growth, yield and nutrients uptake as the function of mycorrhizal inoculation (Ardakani *et al*, 2011).

It is worthy to mention that the results recorded from using VAM with rock phosphate (insoluble P) on plant growth were similar to those attained with superphosphate treatments (soluble P) (Bolan, 1991, Hammond and Leon, 1992 and Smith and Read, 1997), subsequently using a combination of rock phosphate and mycorrhiza could be practical and promising.

Globe artichoke (*Cynara scolymus*, L) is an important vegetable crop in Egypt because of its nutritive and medical values. The immature flower bud (head) is the edible part of the crop which includes the fleshy receptacle and fleshy tender basis of bracts. It is a good source of inulin, fibers and minerals (Ryder *et al*, 1983). The edible flower buds and other

artichoke plant extracts are rich in polyphenols and have high levels of antioxidant activity (Liorachet *et al*, 2002). Moreover, it gained a highly exportable importance to the European markets.

Artichokes seedlings are usually treated with fungicides to shield seedlings from soil disease; however, affect negatively the mycorrhizal growth and activity (Rai, 2006). Thus, finding a new technique for protecting seedlings from soil disease and, at the same time, encourage the mycorrhizal growth is essential. Nanotechnology could be the answer, treating artichokes seedling with nano-silver before planting proved useful in protecting seedling treated with mycorrhiza (*Glomus mosseae*) from disease and in the same time not affecting mycorrhiza activity because this species was not sensitive to nanosilver. (Abbasian *et al*, 2012).

Nanosilver is a new class of materials with remarkable physiochemical and biological characteristics (Choi *et al*, 2009) i. e. antimicrobial activity, antifungal and antiviral effects (Nomiyae *et al*, 2004; Sondi and Salopek-Sondi, 2004) and also can reduce the damages and wounds caused by these diseases (Russell & Hugo, 1994). The nanosilver products make broad claims about the power of their nanosilver ingredients, such as: “eliminates 99% of bacteria” renders material “permanently antimicrobial and antifungal”, “kills approximately 650 kinds of harmful germs and viruses” and “kills bacteria in a short time as 30 min” and it was 2-5 times faster than other forms of silver (Emtiaziet *et al*, 2009).

Global Artichoke can benefit from antimicrobial effect of nanosilver before culture in the field. No published researches were found on the effects of nanosilver on the production efficiency of global artichoke under the field conditions.

The aim of the current research is to find out the feasibility of using rock phosphate as an alternative source of P with or without inoculation with Vesicular Arbuscular Mycorrhizas (VAM) on the growth performance, yield and yield components of artichoke plants treated with the anti-fungal stumps or nano-silver. The inorganic soluble calcium super phosphate was used was also used in this study for result comparison.

MATERIALS AND METHODS

Materials of Study

Rock phosphate (27% P_2O_5) was obtained from Abu Zaabal fertilizer and chemical Co, Egypt. Mycorrhiza (*Glomus mosseae*) were prepared by the Biofertilization Unit, Faculty of Agriculture, Ain Shams University, Egypt. Nanosilver was provided by Nanotech Egypt for Photoelectronics, Bahgat group, 6 October region, Giza Governorate. Surface soil samples (0-30 cm) were collected from the experimental farm of the Faculty of Agriculture, Moshtohor, Benha University, Qalubia Governorate, Egypt prior to each growing season and analyzed for their physical and chemical properties as outlined by Jackson (1969). Physical and chemical properties of the investigated soil are shown in Table 1.

Table 1: Physical and Chemical Properties of the Soil under Study before Transplanting

Soil Texture				pH	EC (dSm ⁻¹)	O.M (%)	CaCO ₃ (%)	Soil Available Macronutrients (Mg Kg ⁻¹)		
Sand (%)	Silt (%)	Clay (%)	Texture					N	P	K
24.4	24.6	51	Clay loam	7.9	2.16	1.41	1.53	22.5	9.1	120

The Field Study

A field experiment was carried out at the experimental Farm of the Faculty of Agriculture, Moshtohor, Benha University, Egypt (www.fagr.bu.edu.eg) during two successive seasons of 2010/2011 and 2011/2012.

Old crowns of globe artichoke (*Cynara scolymus* L.) cv. Fransawy (taken from the previous plants) were used as plant materials for propagation and planted in middle of August during the two seasons of study (2009/2010 and 2010/2011). The experimental plot included four ridges, 1 m width and 4.0 m length with an area of 16 m² from which, three ridges were planted and one was left without planting as a guard between plots. The old crowns were planted at a distance of 1 m apart on one side.

The treatments of study were

- Calcium superphosphate + Anti-fungi* (Control)
- Calcium superphosphate + Nano-silver
- Rock phosphate + Anti-fungi*
- Rock phosphate + Nano-silver
- Rock phosphate + Mycorrhiza
- Rock phosphate + Mycorrhiza + Anti-fungi*
- Rock phosphate + Mycorrhiza + Nano-silver

*Anti-fungi mixture (fungicides) i.e. Reyzolex plus Topsen plus Redomilat 3:2:1.5 g/L for each of them, respectively for 30 min.

The recommended dose of Nano-silver (< 50 nm), was 50 mg/L (Tahmasbi *et al*, 2011). VAM was added at a rate of 1200 mL/ha. One month after transplanting.

The usual cultural practices of the globe artichoke were followed i.e. NPK were added at rates of 600 kg ammonium sulphate (20.6 % N), 150 kg P₂O₅ (either calcium superphosphate or rock phosphate) and 240 kg potassium sulphate (48 % K₂O) per hectare added at three equal doses after 2, 3 and 4 months after planting. This experiment was set out in a complete randomized blocks design with three replicates for each treatment.

Data Recorded

• Vegetative Growth Characters

Five plants were taken from each experimental plot at the beginning of blooming stage (120 days after planting) for measuring the following vegetative growth aspects:-

- Plant height (cm) from the soil surface to the tip of the largest linear blade in plant.
- Number of leaves per plant.
- Leaf area was determined using a digital leaf area meter (L1-300 portable area meter produced by L1-COR, Lincoln, Nebraska, USA).

- At the end of harvesting number of offshoots /plant was accounted.
- **Early and Total Yield**

The early yield was calculated from the start of harvest (after 4 month from transplanting) until the end of February and total yield was up to the end of May (end of the production season of artichoke).

- **Head Quality**
- **Physical Characters**

Five heads were taken randomly from each plot during every harvest period (early – late) in both seasons of study for measuring the head height, head diameter, head fresh weight and the average edible part fresh weight.

- **Chemical Constituents**

Samples of the edible parts of heads were taken at the beginning and the end of harvesting season and dried in an electric oven to constant weight at 70 °C. Inulin concentration was determined according to Winton and Winton (1958) while fibers were determined according to A.O.A.C. (1990).

Statistical Analysis

Statistical analysis was performed using the SPSS package program version 15.0 (SPSS Inc, Chicago, III, USA). Data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range post-hoc test. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSIONS

- **Vegetative Growth**

Data presented in Table 2 show that treating stumps with Nano-silver (NS) improved the growth parameters of plants especially the leave area/plant compared with the Anti-fungal (AF) treatment. It is reported that nano-silver has antibacterial, antifungal and antiviral effects (Nomiya *et al*, 2004; Sondi and Salopek-Sondi, 2004). Probably, VAM symbiosis improved soil structure and protect host plants against the detrimental effects caused by the drought stress, which happens after the harvest season (Schreiner *et al*, 1997). Moreover, the combination between RP + VAM + NS caused further significant increases in the number of offshoots and leave area per plant exceeding those attained by the control combination treatment SP+AF in both seasons. It is worthy to mention that the growth parameters recorded by RP + VAM + AF treatment were significantly similar to those attained by SP+AF. Such results indicate that VAM was not affected by treating stumps with NS, but showed enhancing effect on plant growth comparing to treating with AF compounds as antifungal treatment.

The superiority of Nano-silver than Anti-fungal especially in the case of using rock phosphate and inoculation with VAM (*Glomus mosseae*) may be related to the sensitivity of the VAM species to nanosilver. (Abbasian *et al*, 2012).

The application of traditional fungicide probably damages external hyphae of mycorrhiza and prevents root colonization; reducing water and nutrient uptake, photosynthesis and plant growth (Kung'u *et al*, 2008).

Table 2: Effects of Nano-Silver Versus Traditional Anti-Fungal Stumps on the Plant Growth Parameters of Globe Artichoke Supplied with Either Calcium Super Phosphate or Rock Phosphate as P Sources under Inoculation with Vesicular Arbuscular Mycorrhizas (VAM), during 2010/2011 and 2011/2012 Seasons

Treatments	Plant Height (Cm)		No. of Leaves /Plant		No. of Offshoots / Plant		Leave Area Cm ²	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
Calcium superphosphate + Anti-fungal (control)	87.3 ab	85.0 ab	34.7 abc	36.0 ab	2.67 ab	1.67 c	515 c	519 d
Calcium superphosphate + Nano-silver	94.3 a	93.3 ab	39.7 ab	41.6 a	3.03 ab	3.01 ab	526 b	532 c
Rock phosphate + Anti-fungal	80.0 b	82.6 b	31.7 c	30.0 b	0.67 c	1.33 C	495 d	492 e
Rock phosphate + Nano-silver	84.3 ab	83.4 b	33.7 bc	31.6 ab	2.33 b	1.67 c	516 c	518 d
Rock phosphate + Mycorrhiza	87.0 ab	87.3 ab	37.0 abc	35.7 ab	2.67 ab	2.33 abc	531 ab	535 b
Rock phosphate + Mycorrhiza + Anti-fungal	89.7 ab	91.0 ab	33.3 bc	36.7 ab	2.23 b	2.01 bc	514 c	519 d
Rock phosphate + Mycorrhiza + Nano-silver	95.0 a	96.0 a	41.0 a	42.3 a	3.33 a	3.33 a	536 a	540 a

Means of the same column followed by the same letter were not significantly different according to Duncan MRT at 5%.

Early and Total Yield

Table 3 reveal that the nano-silver treatments were more preferable than the antifungal treatments on increasing the total yield of globe artichoke. On the other hand, RP+VAM recorded higher increases in the total yield than those attained by SP treatments; however such increases seemed to be insignificant in most cases. Similar results indicate the improvement of plant growth, yield and nutrients uptake as the result of mycorrhizal inoculation (Akond *et al*, 2008, Ardakani *et al*, 2011, Abbasian *et al*, 2012). The activity of mycorrhiza external hyphae is related to succinate dehydrogenase enzyme which improves plant photosynthesis and P uptake (Gyaneshwar *et al*, 2002, Xiao *et al*, 2008). The combination between NS, RP and VAM recorded the highest increases in total yield exceeding the treatment which received RP + VAM + AF and the control one SP+AF in both seasons. This results was confirmed by Abbasian *et al*. (2012) who found that VAM was not sensitive to nano-silver.

Table 3: Effect of Calcium Super Phosphate or Rock Phosphate Only or with Inoculation by Vesicular Arbuscular Mycorrhizas (VAM) on Early, Total Yield and its Components of Globe Artichoke which was treated with Anti-Fungal Stumps or Nano-Silver, during 2010/2011 and 2011/2012 Seasons

Treatments	Early Yield (Ton/Ha)		Total Yield (Kg/Plant)		Total Yield (Ton/Ha)	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
Calcium superphosphate + Anti-fungal (control)	5.58 bc	5.79 bc	3.47 b	4.15 a	32.71 c	39.43 c
Calcium superphosphate + Nano-silver	7.79 ab	9.79 ab	4.09 a	4.47 a	38.71 b	42.10 b
Rock phosphate + Anti-fungal	1.51 d	1.31 d	2.60 c	2.60 c	24.52 e	24.62 f
Rock phosphate + Nano-silver	2.84 cd	2.86 cd	2.77 c	2.86 c	26.31 e	26.67 e
Rock phosphate + Mycorrhiza	7.68 ab	8.90 ab	3.38 b	3.62 b	31.95 c	34.17 d
Rock phosphate + Mycorrhiza + Anti-fungal	7.02 ab	6.62 abc	3.10 b	3.95 b	29.29 d	37.33 c
Rock phosphate + Mycorrhiza + Nano-silver	9.99 a	10.46 a	4.36 a	4.77 a	41.33 a	45.26 a

Means of the same column followed by the same letter were not significantly different according to Duncan MRT at 5%.

Head Quality

• Head Diameter and Head Length

Data shown in Table 4 indicate that the combined treatment RP, VAM and NS recorded the highest physical quality traits (Head diameter and length in early and late yield) with significant deference comparing with the control treatment SP + AF in both seasons. On the other hand, the lowest records of physical quality properties were observed in with the treatment

RP and AF.

Table 4: Effect of Calcium Super Phosphate or Rock Phosphate Only or with Inoculation by Vesicular Arbuscular Mycorrhizas (VAM) on early and Late Heads Characteristics of Globe Artichoke Which was Treated with Anti-Fungal Stumps or Nano-Silver, during 2010/2011 and 2011/2012 Seasons

Treatments	Early Yield				Late Yield			
	Head Diameter (Cm)		Head Length (Cm)		Head Diameter (Cm)		Head Length (Cm)	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st season	2 nd season
Calcium superphosphate + Anti-fungal (control)	7.57 bc	7.63 bc	11.50 a	11.83 a	6.57 c	6.77 b	11.03 b	11.20 cd
Calcium superphosphate + Nano-silver	8.60 ab	9.10 ab	12.17 a	12.00 a	7.68 b	7.03 a	12.00 a	12.03 ab
Rock phosphate + Anti-fungal	7.13 c	7.43 c	11.33 a	11.17 a	6.13 d	6.37 b	10.62 c	10.97 d
Rock phosphate + Nano-silver	7.67 bc	7.83 bc	11.00 a	11.50 a	6.69 c	7.74 b	11.00 b	11.49 bc
Rock phosphate + Mycorrhiza	8.53 ab	8.83 abc	12.17 a	12.03 a	7.57 b	7.76 a	12.03 a	11.85 ab
Rock phosphate + Mycorrhiza + Anti-fungal	8.00 abc	7.80 bc	11.67 a	12.00 a	6.82 c	6.80 b	11.13 b	11.80 ab
Rock phosphate + Mycorrhiza + Nano-silver	9.10 a	9.00 a	12.23 a	12.43 a	8.27 a	7.87 a	11.89 a	11.83 a

Means of the same column followed by the same letter were not significantly different according to Duncan MRT at 5%

Fresh Weights of the Edible Parts and Heads of Globe Artichoke

Data presented in Table 5 show that plants treated with either RP+VAM+NS or SP+NS, improved the fresh weight of the edible part than the control SP + AF treatment. This trend was true in both seasons. It seems that the silver nano-particles product was effective in increasing the fresh weights of the edible parts and heads of globe artichoke than the traditional anti-fungi stump. Moreover, rock phosphate application under inoculation with VAM can effectively substitute the traditional chemical calcium super phosphate without affecting the yield components of globe artichoke.

Table 5: Effect of Calcium Super Phosphate or Rock Phosphate Only or with Inoculation by Vesicular Arbuscular Mycorrhizas (VAM) on Early and Late Heads Characteristics of Globe Artichoke which was treated with Anti-Fungal Stumps or Nano-Silver, during 2010/2011 and 2011/2012 Seasons

Treatments	Early Yield				Late Yield			
	Average Head Fresh Weight (G)		Average Edible Part Fresh Weight (G)		Average Head Fresh Weight (G)		Average Edible Part Fresh Weight (G)	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
Calcium superphosphate + Anti-fungal (control)	243.3 ab	238.3 a	59.0 b	45.7 c	238.3 a	231.8 bc	45.5 d	43.6 d
Calcium superphosphate + Nano-silver	256.7 a	261.7 a	66.3 a	70.7 a	243.4 a	241.0 ab	65.3 b	69.4 b
Rock phosphate + Anti-fungal	195.0 c	190.6 b	35.3 c	35.3 d	183.6 c	182.9 d	30.4 g	29.6 e
Rock phosphate + Nano-silver	199.0 bc	203.3 b	45.7 b	46.7 bc	191.0 c	190.0 d	42.7 e	44.3 d
Rock phosphate + Mycorrhiza	250.0 a	246.7 a	53.0 b	60.0 b	227.7 b	228.3 c	60.4 c	59.2 c
Rock phosphate + Mycorrhiza + Anti-fungal	246.7 a	241.7 a	50.0 b	51.7 bc	223.5 b	235.0 bc	41.9 f	44.4 d
Rock phosphate + Mycorrhiza + Nano-silver	276.7 a	272.0 a	73.3 a	74.0 a	251.5 a	254.7 a	70.6 a	71.5 a

Means of the same column followed by the same letter were not significantly different according to Duncan MRT at 5%.

Inulin and Fiber Contents of Globe Artichoke Heads

Data in Tables 6 indicate that the highest inulin contents and lowest fiber contents in edible parts was recorded for plants treated with SP + NS and RP + NS + VAM with no significant differences between these two treatments. Probably VAM affect the plant secondary metabolism producing healthy promoting activities, thus improved the quality of the flower heads of artichoke plants (Ceccarelli *et al*, 2010). On the other hand, the highest fiber content and the lowest inulin content were found in plants that received RP +AF. Although, a negative relation probably exists between inulin and fiber contents in the globe artichoke heads; however, both of them are beneficial for man use. Inulin is a plant-driven carbohydrate which has the benefits of soluble dietary fiber (López-Molina *et al*, 2005). It increases absorption of Ca and Mg in blood, promotes the blood glucose

formation and reduce the level of cholesterol (Niness, 1999). High contents of artichoke fiber in wheat breads could improve the textural and sensory qualities of bread (Frutos *et al.*, 2008). Fibers can also be used as potential reinforcement of composite structures (Fiore *et al.*, 2011).

Treatments on head quality are connected with those effects on increasing vegetative growth plant growth (Table 2). These results were in agreement with Ardakani *et al.* (2011), Nedorost and Pokluda (2012) and Abbasian *et al.* (2012) who found that improvement of plant growth by mycorrhizal inoculation leads to improved quality specifications.

Table 6: Effect of Calcium Super Phosphate or Rock Phosphate Only or with Inoculation by Vesicular Arbuscular Mycorrhizas (VAM) on Inulin and Fiber Contents of Globe Artichoke Heads which was treated with Anti-Fungal Stumps Or Nano-Silver, During 2010/2011 And 2011/2012 Seasons

Treatments	Early Yield				Late Yield			
	Inulin %	Fiber Contents (Mg/G Dw)	Inulin %	Fiber Contents (Mg/G Dw)	Inulin %	Fiber Contents (Mg/G Dw)	Inulin %	Fiber Contents (Mg/G Dw)
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
Calcium superphosphate + Anti-fungal (control)	1.63 b	1.62 c	4.48 abc	4.50 bc	1.40 ab	1.32 bc	6.00 cd	6.03 c
Calcium superphosphate + Nano-silver	1.81 a	1.83 ab	4.42 bc	4.49 bc	1.42 ab	1.43 ab	5.82 d	5.80 c
Rock phosphate + Anti-fungal	1.47 c	1.53 c	4.60 a	4.69 a	1.32 bc	1.26 c	8.27 a	8.20 a
Rock phosphate + Nano-silver	1.52 bc	1.57 c	4.57 ab	4.57 abc	1.34 bc	1.34 abc	7.30 b	7.38 b
Rock phosphate + Mycorrhiza	1.58 bc	1.59 c	4.50 abc	4.58 ab	1.38 ab	1.34 abc	5.90 cd	5.93 c
Rock phosphate + Mycorrhiza + Anti-fungal	1.65 b	1.68 bc	4.53 abc	4.50 bc	1.24 c	1.27 c	6.10 c	6.19 c
Rock phosphate + Mycorrhiza + Nano-silver	1.88 a	1.90 a	4.38 c	4.42 c	1.50 a	1.49 a	5.83 d	5.85 c

Means of the same column followed by the same letter were not significantly different according to Duncan MRT at 5%.

CONCLUSIONS

Application of rock phosphate, RP as a source of P together with inoculation by Vesicular Arbuscular Mycorrhizae is a recommended agriculture practice to increase the vegetative growth, early and total yield of globe artichoke. This treatment resulted in higher growth parameters and better yield quantity and quality than those attained with application of calcium superphosphate fertilizers. Moreover, the addition of RP + VAM decreases manufacturing costs for producing SP as artificial fertilizer. On the other hand, pre-planting treatment propagation material with Nano-silver is the recommended antifungal treatment to obtain the highest head flower yield of Globe artichoke with the best quality without showing negative effects on the symbiotic mycorrhizae (*Glomus mosseae*).

ACKNOWLEDGEMENTS

The authors would like to present deep gratefulness and appreciation to Dept. of Horti, Fact. of Agric, Benha University, Egypt (www.bu.edu.eg) for giving them the opportunity to fulfil this study.

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